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DIVISION S-4—SOIL FERTILITY & PLANT NUTRITION

Contributions of Shoot and Root Nitrogen-15 Labeled Legume Nitrogen Sources to a Sequence of Three Cereal Crops

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ABSTRACT

Legume mulches are important sources of N for cereal crop production, particularly for organic and resource-poor producers. A field study was conducted using a direct method to determine if the amount of N in cereal crops derived from either the shoots or roots of preceding tropical legume cover crops was affected by their chemical composition and mineralization potential. *Desmodium ovalifolium* Guill. & Perr. [= *D. adscendens* (Sw.) DC. and *Pueraria phaseoloides* (Roxb.) Benth.], were grown in 6.0-m^2 microplots and foliar-labeled with 99 atom % ^{15}N urea. A cereal sequence of maize (*Zea mays* L.)–rice (*Oryza sativa* L.)–maize followed the legumes. Cereal accumulation of legume N from either the shoot (shoot + leaf litter) or the root-soil sources was evaluated by spatially separating the legume N sources. This was achieved by interchanging surface applications of nonlabeled and ^{15}N-labeled legume shoots with in situ ^{15}N-labeled and nonlabeled legume roots. Initially the *Desmodium* shoot N source contained 316 kg N ha^{-1} and roots contained 12.5 kg N ha^{-1}. *Pueraria* shoots and root N sources initially contained 262 and 14.8 kg N ha^{-1}, respectively. About 90 g kg^{-1} of the initial N of each legume shoot was recovered in the total aboveground tissues from the three cereal crops, while 490 g kg^{-1} of *Desmodium* and 280 g kg^{-1} of *Pueraria* root-soil N sources were recovered. Of the 181 kg N ha^{-1} accumulated aboveground by the cereal sequence, the contribution of shoot plus root-soil N sources was 200 g kg^{-1} from *Desmodium* and 150 g kg^{-1} from *Pueraria*. Cereal N was derived primarily from mineralization of soil organic matter present before the legumes and possibly from N deposition (precipitation and dry) occurring during the cereal crop sequence. After harvest of the last cereal crop, 13 and 180 g kg^{-1} of the initial legume N was present as inorganic and organic N fractions, respectively, in the top 75 cm of soil. Even though *Pueraria* shoots had a lower C:N ratio and concentration of polyphenols than *Desmodium* shoots, the relative contributions of the shoot N source were similar for both legumes. Decomposition of legume residues, particularly legume shoots, make a meaningful contribution to the N economy of cereal crops grown in the tropics. The legume cover crops (root + shoot) contributed nearly 280 g kg^{-1} of the aboveground N in the first cereal crop and as much as 110 g kg^{-1} of the N in the third crop during the 15-mo sequence of cereals.

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Legumes are used commonly in agricultural systems as a source of N for subsequent crops and for maintaining soil N levels. This use is particularly important in the humid tropics where N fertilizers often are not economically feasible due to poor market and infrastructure development (Palm and Sanchez, 1991). To date, studies attempting to quantify the legume N contribution to subsequent crops have been conducted mainly in temperate agroecosystems and have dealt primarily with aboveground legume N, ignoring root N because of the difficulty of harvesting roots and nodules. Moreover, these assessments of N cycling in cover crop based production systems have often relied on indirect methods that evaluate plant and soil N pools (Ditsch et al., 1993; Luna-Orea and Wagger, 1996), N release from cover crop residue (Ranells and Wagger, 1991; Luna-Orea et al., 1996), and N uptake by a summer crop (Hargrove, 1986; Clark et al., 1994).

Nitrogen-15 methodology is useful for resolving N dynamics, whereby ^{15}N-labeled legume cover crops are harvested and applied as N sources for subsequent grain crops (Varco et al., 1989; Jordan et al., 1993; Harris et al., 1994). Varco et al. (1993) found that 600 g kg^{-1} of the N was mineralized and subsequently lost from ^{15}N-labeled hairy vetch (*Vicia villosa* Roth) residue 30 d after surface application, yet an average of only 60 g kg^{-1} was recovered as soil inorganic N for two growing seasons. In Australia, Ladd and associates (Ladd et al., 1981, 1983; Ladd and Amato, 1986) reported field-grown wheat (*Triticum aestivum* L.) recovered between 11 and 280 g kg^{-1} N from ^{15}N-labeled medic (*Medicago littoralis* L.) and an additional 40 g kg^{-1} recovery by a second wheat crop. When ^{15}N-labeled red clover (*Trifolium pratense* L.) residue was applied at maize planting, 150 g kg^{-1} was recovered in the harvested crop and 570 g kg^{-1} was retained by the soil (Harris et al., 1994). Of these studies, that of Varco et al. (1989) made an indirect estimate of the contribution of legume root N to subsequent crops. Only a few direct estimates of N contribution from legume shoot and root residues are available (Harris and Hesterman, 1990; Russell and Fillery, 1996).

The objectives of this study were to: (i) quantify the N contribution from two tropical legume cover crops of differing chemical characteristics (i.e., potentially dif-

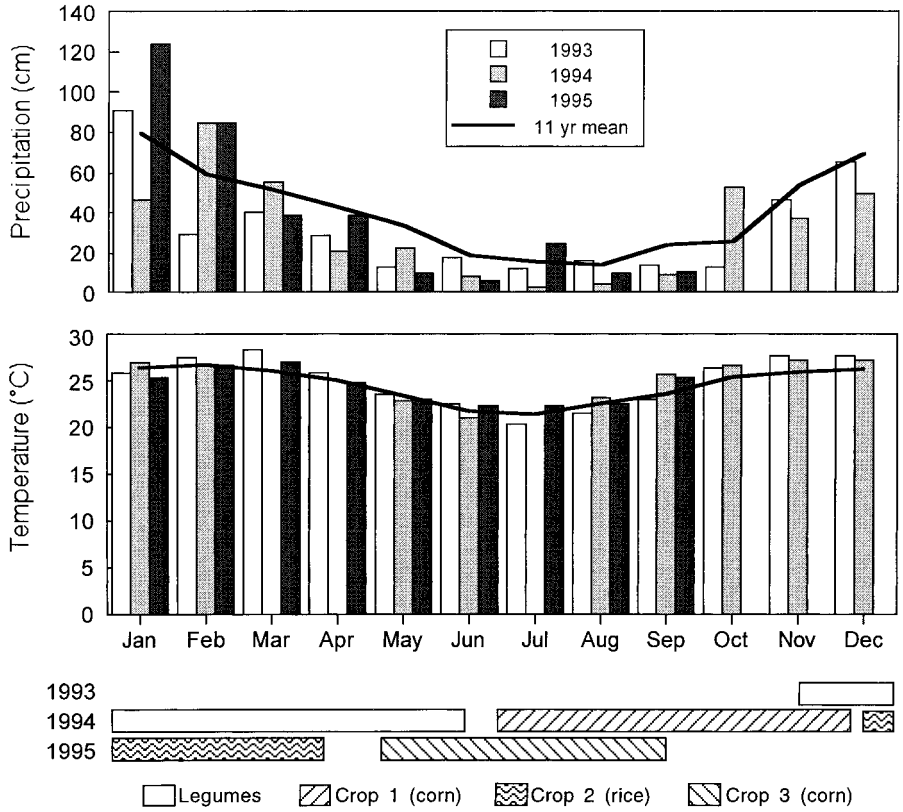

Fig. 1. Monthly and 11-yr mean (1982–1993) precipitation and temperature at La Jota Experiment Station, Bolivia, 1993 to 1995. Temperature not recorded during March and April 1994. Horizontal bars indicate crop duration of legumes and cereal crops.

ferent rates of decomposition and nutrient release) to a subsequent maize–rice–maize sequence using ^{15}N methodology, (ii) determine the effect of plant part (shoot vs. root) on recovery of legume-derived ^{15}N by grain crops, and (iii) quantify the legume-derived ^{15}N remaining in various soil N fractions at the end of the cereal crop sequence.

MATERIALS AND METHODS

A field study was conducted at La Jota Research Station (16°01′ S and 65°25′ W; 400 m above sea level), situated in the sub-Andean foothills of eastern Bolivia, on a gently sloping (0–2%) fine-loamy, mixed, isohyperthermic, Typic Dystropepts. Selected soil physical and chemical characteristics of the surface 20 cm are as follows: 41% sand, 41% silt, 18% clay, bulk density 1.02 Mg m^{-3}, 13.8 g C kg^{-1}, 1.8 g N kg^{-1}, effective CEC 7.0 $cmol_c$ kg^{-1} (1 *M* NH_4OAc, pH 7; 1 *M* KCl-extractable Al), 880 g kg^{-1} Al saturation, and pH 4.6 (1:1, soil:water). Rainfall and temperature data for the site, covering the 21-mo study period, are presented in Fig. 1.

Cover Crop Establishment

From June through October 1993, a 1 ha, 6- to 19-yr-old secondary forest was cut and all aboveground vegetation was carried off the experimental site and burned. Negligible litter remained on the soil surface, which was essentially undisturbed. The top 75 cm of soil contained 98 kg N ha^{-1} as inorganic N just before the legume cover crops were planted. In mid-November, 10- by 15-m plots of two tropical legumes [*D. ovalifolium* and *P. phaseoloides* (tropical kudzu)] and fallow check were established and randomized in each of four blocks. *Desmodium* and *Pueraria* seed were coated with a sugar-water sticker, inoculated with CIAT 4099 and CIAT 3287 *Rhizobia*, respectively, pelleted with 100 g of $CaCO_3$ (powder) per kg of seed, and planted in 50-cm wide rows at a seeding rate of 27 kg ha^{-1}. During an 8-mo growth period, legumes were maintained weed free by hand and pest free with applications of malathion (O,O-dimethyl phosphorodithioate of diethyl mecaptosuccinate). Weeds were allowed to grow in the fallow plots during the 8-mo legume growth period. At termination of legume cover crop growth, weeds in the fallow plot were cut and left on the soil surface.

Cover Crop Nitrogen-15 Labeling

In mid-December (early in legume development to minimize root damage), two 6-m^2 microplots containing five legume rows were sectioned off within each plot using galvanized steel collars (20 cm high) driven into the soil ≈15 cm to reduce lateral flow of water and ^{15}N. Sixteen microplots were installed, eight for each species representing two ^{15}N sources and four replications. Within each legume plot, one of the microplots was randomly selected for foliar treatment with ^{15}N. Shoots from the ^{15}N-labeled microplot were exchanged later with shoots from the adjacent unlabeled microplot to spatially separate ^{15}N-labeled root from ^{15}N-labeled shoot sources of legume N for the following maize–rice–maize sequence.

Nitrogen-15 was applied to growing legumes beginning in mid-May 1994 when *Desmodium* shoot dry matter accumulation was 9.7 Mg ha^{-1} (184 kg N ha^{-1}) and *Pueraria* was 4.2 Mg ha^{-1} (144 kg N ha^{-1}). The soil surface beneath the legume canopies was not visible. *Desmodium* and *Pueraria* began flowering just prior to initiation of ^{15}N foliar labeling. To prevent

seed set and to minimize N translocation from roots to shoots, flowers were clipped during the ^{15}N labeling period, dried, and later combined with the shoot ^{15}N source. A total of 2.58 g ^{15}N (4.3 kg N ha^{-1}) as urea labeled with 99 atom % ^{15}N was applied to each microplot in four equal foliar applications of 0.645 g ^{15}N each (18 and 30 May and 6 and 13 June). A 12-d interval occurred between the first and second ^{15}N applications because of rainy conditions. Each microplot was divided into quadrants to assure uniform distribution of the ^{15}N solution sprayed on the upper plant canopy using a hand-held mister. The solution contained 300 mL H_2O (volume necessary to maximize foliar coverage while minimizing drip, potential ^{15}N loss, and soil contamination), a wetting agent to maximize absorption, and the ^{15}N-labeled urea. Immediately following each foliar application, microplots were covered with a transparent plastic shelter for 2 d to prevent rainfall from washing ^{15}N off the leaves.

Sampling and Analysis of Nitrogen-15-Labeled Microplots

Ten days after the final ^{15}N foliar split-application, legume shoots were cut at the soil surface and removed and then leaf litter was collected. A subsample of shoots was weighed, air-dried, ground to 1 mm, and analyzed for polyphenolic and lignin concentrations using the procedure described by Palm and Sanchez (1991) and Van Soest (1963), respectively. The remaining shoots and leaf litter were weighed, subsampled for moisture determination, oven dried at 65 °C, reweighed, and ground to 1 mm. Obtaining representative subsamples is frequently a problem with ^{15}N field-tracer studies; thus the oven-dried and ground plant subsamples were thoroughly mixed in a twin-shell blender, and 5-g subsamples were ground to a fine powder in a dental amalgam ball mill. Finally, plant subsamples (≈5 mg) containing at least 100 μg N were weighed into Sn capsules for total N and ^{15}N determinations with an automated flash-combustion analyzer coupled to an isotope ratio mass spectrometer (RoboPrep and TracerMass, Europa Scientific, Crewe, Cheshire, UK).[1]

The present study was designed to leave the labeled roots undisturbed and to follow the recovery of ^{15}N from the combined root-soil system by subsequent crops. Immediately following removal of legume shoots, four soil cores were taken at five depths (0–10, 10–25, 25–40, 40–60, and 60–75 cm), air-dried, and sieved (2mm). Obtaining representative soil subsamples is often a problem with ^{15}N field-tracer studies, more so than with plants due to the inherent variability of soil N. Thus the air-dried, sieved root-soil samples were thoroughly mixed in a twin-shell blender and 5-g subsamples were ground to a fine powder in a dental amalgam ball mill. Finally, subsamples of root-soil (≈40 mg) containing ≈25 to 80 μg N were weighed into Sn capsules for total N and ^{15}N determinations with an automated flash-combustion analyzer coupled to an isotope ratio mass spectrometer.

Inorganic Soil Nitrogen and Nitrogen-15 Analysis

Air-dried and sieved soil samples were shaken with 2 *M* KCl (20 g soil 100 mL^{-1}) for 1 h, filtered, and analyzed for total inorganic N ($NH_4 + NO_3$) with an automated flow injection ion analyzer (QuickChem IV, Lachat Instruments, Milwaukee, WI). Inorganic N was isolated for ^{15}N analysis by the method of Brooks et al. (1989) as follows. An extract volume containing 60 to 100 μg total N was transferred into a 104-mL specimen cup, and 0.4 g MgO, 0.2 g of Devarda's alloy (ground to a powder with a ball-mill), and 7.0 g K_2SO_4 (to increase solution osmotic potential and reduce H_2O vapor pressure) were added. A 6-mm diameter acid-washed filter paper disk, acidified with 10 μL of 2.5 *M* $KHSO_4$, was suspended above the extract-reagent mixture to trap volatilized NH_3. The cup was capped, gently swirled, and incubated at 40 °C for 6 d. Following incubation, the paper disk was removed, dried in a vacuum microcentrifuge, wrapped in a Sn capsule and analyzed for ^{15}N. The soil organic ^{15}N fraction was determined by subtracting the inorganic ^{15}N from total ^{15}N.

Cereal Cropping Sequence

On 24 June 1994, the harvested unlabeled and ^{15}N-labeled legume shoots and litter were used to establish microplots for the cereal cropping sequence. Within each replicate, ^{15}N-labeled shoots and litter from the original foliar ^{15}N-labeled microplot were removed and surface-applied to an adjacent microplot containing only unlabeled roots, that is, legumes in this new microplot had not received the foliar ^{15}N application. Then, an equal weight of shoots plus litter (means for *Desmodium* and *Pueraria* were 19.2 and 8.6 Mg ha^{-1}, respectively) from this unlabeled legume microplot were surface-applied to the microplot containing only the ^{15}N-labeled root-soil N source. After redistributing surface residue, the microplots were left undisturbed for 7 d before annual crops were planted. Legumes outside the microplots were cut and left on the soil surface as mulch.

Maize ('Across 8136'), the first cereal crop of the sequence, was sowed 30 June 1994 (≈30 700 plants ha^{-1}) using a jab planter and a 65- by 50-cm grid pattern within the microplots, the border area between microplots, and the fallow check plots. Post-emergence weed control and *Desmodium* regrowth proved challenging throughout the first maize crop, necessitating bi-weekly applications of 1 kg a.i. ha^{-1} of paraquat [1, 1'-dimethyl-4 4'-bipyridinium ion (dichloride salt)] in combination with hand weeding. Pests, particularly a severe *Spodoptera frugiperda* (J.E. Smith) infestation triggered by a 10-yr record drought, were controlled with weekly sprayings of methamidophos [O,S-dimethyl phosphoramidothiate] and malathion. Approximately 6 wk after planting the first maize crop, all microplots were fertilized by hand with 29, 111, 21, and 16 kg ha^{-1} of P, K, Ca, and Mg, respectively. Fertilizer trials had not been conducted in this region, so fertilizer tests for macronutrients were made and applications were conservatively calculated as twice the nutrient removal from an average local maize and rice yield of 2.3 and 2.9 Mg ha^{-1}, respectively. Aboveground parts of mature maize plants were harvested on 7 Dec. 1994 from each microplot. Grain and stover were weighed, and subsampled for moisture, total N, and ^{15}N determinations. Grain yield was adjusted to a 155 g kg^{-1} moisture basis. After removal of the maize plants an equal weight of unlabeled maize stover was surface-applied to the microplots.

Upland rice ('Bluebell') followed maize in the cereal sequence and was planted 15 Dec. 1994 (≈100 000 plants ha^{-1}) on a 40- by 25-cm planting grid with a jab planter. Before rice emergence, the area was sprayed with paraquat (1 kg a.i. ha^{-1}) to kill existing vegetation. As with the maize crop, post-emergent weed control and *Desmodium* regrowth proved challenging, requiring five hand cultivations. Additionally, weekly spray applications of malathion were necessary to control various insect stresses. Fertilization rates for all nutrients were doubled for the rice crop as visual P deficiencies were observed in ≈10% of the maize plants. Aboveground parts of mature rice plants were harvested 7 Apr. 1995 from each

[1] The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Research Service or USDA-ARS of the product named, nor criticism of similar ones not mentioned.

microplot, rice grain and straw were weighed, and subsampled for moisture, total N, and ^{15}N determinations. The labeled rice straw was then returned to the respective microplots, while all grain was removed from the field and oven-dried at 65 °C for 24 h. Rice yields were not adjusted to a specific moisture basis due to logistical constraints, though local experience indicates oven drying at 65 °C for 24 h yields an approximate moisture basis of 80 g kg^{-1}.

Maize followed rice as the last crop in the cereal sequence and was planted 3 May 1995. Cultural practices were the same as previously described for the first maize crop, with the exception of doubling the original fertilization rate of the first maize crop as detailed above. At maturity the aboveground parts of the last maize crop were harvested on 15 Sept. 1995 and processed as previously described for the first crop of maize in the cereal sequence.

Soil sampling was conducted at the end of each cereal crop season as previously described. Additionally, tissue, grain, and soil samples were analyzed for total N and ^{15}N enrichment as previously described. Total plant C was determined with a CHN elemental analyzer (Model 2400, Perkin Elmer, Norwalk, CT).

Recovery Calculations and Statistical Analyses

Recoveries of foliar-applied ^{15}N-urea were calculated using the sum of atom % excess ^{15}N in the shoot plus soil (0 to 75 cm). Recoveries of legume sources of ^{15}N in the following cereal crops were determined after each cereal harvest by measuring the atom % excess ^{15}N in aboveground tissues and the top 75 cm of the soil profile. For the legume root-soil ^{15}N source, it was assumed that the ^{15}N label was contained entirely in the root, even though a portion of the root-soil ^{15}N source was present as inorganic N before planting the first cereal crop. The amount of N contained in the legume roots of each ^{15}N-labeled microplot was assumed to be proportionally equal to that measured following excavation of legume roots in an identical ^{15}N labeling experiment (Glasener et al., 1998) that was conducted simultaneously. Consequently, the recoveries from this source would represent an upper limit for N derived entirely from legume roots.

The experiment was a split plot design with four replications. Each legume main plot had two randomized subplots consisting of the legume root and shoot ^{15}N sources contained in microplots. Nitrogen-15 recovery data was analyzed by legume species, ^{15}N source, and the interaction of these factors using PROC GLM (SAS, 1991). Pre-planned contrasts were implemented to distinguish significant differences among treatment effects.

RESULTS AND DISCUSSION

Legume Cover Crop Traits

In mid-June 1994, when ^{15}N-labeled cover crops were harvested, *Desmodium* had produced ≈2.9-fold more shoot dry matter (17.0 Mg ha^{-1}) than *Pueraria* (5.9 Mg ha^{-1}), and had a N concentration that was only 16 g kg^{-1} compared with 33 g kg^{-1} for *Pueraria* (Table 1).

Table 1. Aboveground dry matter and selected chemical traits of *Desmodium* and *Pueraria* legume cover crops labeled with ^{15}N.

Legume	Dry matter	Total N	Lignin	Polyphenols	C:N ratio
	Mg ha^{-1}	g kg^{-1}			
Desmodium	17.0a†	15.9b	125a	15.3a	25a
Pueraria	5.9b	32.8a	104a	10.6b	13b
CV, %	5	3	6	1.8	5

† Means within a column followed by the same letter are not significantly different ($P \leq 0.05$).

Table 2. Recoveries of foliar-applied ^{15}N-urea in the shoot and root-soil components of microplots grown with *Desmodium* and *Pueraria* legume cover crops.

Legume cover crop	Shoot†	Root-soil (0–75 cm)			Microplot total
		Inorganic	Organic	Total	
	% ^{15}N recovery				
Desmodium	73.8a‡	0.3b	0.9b	1.2b	75.0a
Pueraria	75.8a	0.6a	3.4a	4.0a	79.8a
CV, %	10	22	34	28	10

† Includes leaf litter plus flowers representing recoveries of 3.9 and 7.7% of foliar applied ^{15}N-urea to *Desmodium* and *Pueraria*, respectively.
‡ Means within a column followed by the same letter are not significantly different ($P \leq 0.05$).

Consequently, the C:N ratio of *Desmodium* was ≈2-fold greater than that of *Pueraria*. Lignin concentrations were similar for the two legumes, while the polyphenol concentration of *Desmodium* shoots exceeded that of *Pueraria* by ≈1.5-fold (Table 1).

Recovery of Foliar-Applied Nitrogen-15

About 750 g kg^{-1} of the ^{15}N-urea applied to the canopy of the legume cover crops was recovered in the aboveground tissues (shoots, flowers, and leaf litter) and the top 75 cm of soil in the ^{15}N-labeled microplots. Less than 40 g kg^{-1} of the foliar applied ^{15}N-urea was found in the root-soil N pool, with the organic fraction of the root-soil N pool containing more of the foliar-applied ^{15}N than the inorganic fraction (Table 2). Recoveries of ^{15}N as inorganic, organic, and total N fractions in the soil were greater from *Pueraria* than *Desmodium* microplots. Previous study of ^{15}N labeling of legume canopies produced similar results; the effectiveness of ^{15}N foliar labeling of *Desmodium* and *Pueraria* measured an average recovery of 780 g kg^{-1} in shoots and only 41 g kg^{-1} in the excavated roots and soil (Glasener et al., 1998). Vasilas et al. (1980) also found the leaves and stems of soybean [*Glycine max* (L.) Merr.] to be the dominant sinks for foliar-applied ^{15}N, and no more than 16 g kg^{-1} of the applied N was translocated to the roots. Morris and Weaver (1983) applied ^{15}N-labeled urea to foliage of soybean and recovered an average of 680 g kg^{-1} in the shoots and 17 g kg^{-1} in the roots after three sampling dates. We observed some spray drift beyond the perimeter of the ^{15}N-labeled microplots that would account partially for the unrecovered foliar applied ^{15}N in our study. Additional losses may have occurred as urea was absorbed into the leaf (Wittwer et al., 1963), hydrolyzed, and then volatilized as NH_3.

Within ^{15}N-labeled microplots, total N in the top 75 cm of the soil profile was equivalent (averaging 8250 kg ha^{-1}) for the two legume cover crops (Table 3). Rather than disturb the soil to excavate legume roots to measure their N content, the contribution of legume root N to the soil total N pool of the microplots was calculated using data from our previous research (Glasener et al., 1998) with identical experimental conditions (i.e., same time period, identical growing conditions, same legumes) in which shoot and root dry matter and N content were measured. Estimated root N was 12.5 kg ha^{-1} for *Desmodium* and 14.8 kg ha^{-1} for *Pueraria*

Table 3. Total N and ^{15}N enrichment of microplot N sources supplied by labeled legume shoot and root-soil (0- to 75-cm depth) components and the calculated N content of roots in the ^{15}N-labeled microplots.

Legume cover crop	Total N†	^{15}N enrichment	Estimated root N‡
	kg ha^{-1}	atom % excess	kg ha^{-1}
	Labeled shoot		
Desmodium	316a§	0.990b	–
Pueraria	262b	1.235a	–
CV, %	8	5	–
	Labeled root-soil		
Desmodium	8260a	0.0006b	12.5b
Pueraria	8240a	0.0021a	14.8a
CV, %	11	40	4

† Total N includes leaves, stems, flowers, and leaf litter for shoot N source.
‡ Product of shoot (leaves, stems, and flowers) total N and the root:shoot N ratios of *Desmodium* (0.0462) and *Pueraria* (0.0761) legume cover crops grown simultaneously in another experiment (Glasener et al., 1998).
§ Legume N component means within a column followed by the same letter are not significantly different ($P \leq 0.05$).

(Table 3), much less than the 268 kg N ha^{-1} of *Desmodium* shoots (leaf + stem + flowers) that was 1.4-fold greater ($P \leq 0.05$) than the 190 kg N ha^{-1} of *Pueraria* shoots. Total N supplied by the *Desmodium* shoot source (shoot + leaf litter) was 1.2-fold greater than that supplied by *Pueraria* (Table 3), even though *Pueraria* had more leaf litter N (72 kg N ha^{-1}) than Desmodium (48 kg N ha^{-1}). Because the legume root N was a minor component of the total root-soil N pool, the ^{15}N enrichment of the root-soil pool was <0.003 atom % excess ^{15}N, a value much less than the 0.990 (*Desmodium*) and 1.235 (*Pueraria*) atom % excess ^{15}N of the legume shoot N sources (Table 3).

Soil Inorganic Nitrogen

Total inorganic N in the top 75 cm of the soil profile declined from 98 kg ha^{-1} after removal of aboveground native vegetation and before planting the legumes, to ≈18 kg ha^{-1} during growth of legumes and weeds in the fallow plots (Table 4). The soil inorganic N differential following native vegetation clearing and establishment of the legumes was likely the consequence of mineralization of belowground native vegetation and subsequent N uptake by the legume and weeds present in the fallow plots during cover crop establishment. Following legume establishment, soil inorganic N then appeared to increase soon after cutting aboveground vegetation (legumes or fallow weeds) from the plots and planting the first cereal crop of maize in the ^{15}N-labeled legume and fallow plots. At this time, before the onset of rapid growth of the first cereal crop of maize, inorganic N in plots with *Pueraria* cover crop had 15 kg N ha^{-1} more soil inorganic N than plots with *Desmodium*, and 23 kg N ha^{-1} more than the initially fallow check plots. At the end of each crop in the cereal sequence, no significant differences ($P \leq 0.05$) in the amount of soil inorganic N were observed among the legume cover crop plots and plots that were fallow when the cover crops were grown (Table 4).

The initially greater soil inorganic N of *Pueraria* plots

Table 4. Total inorganic N (NO_3 + NH_4) from 1993 to 1995 in the top 75 cm of soil in microplots planted with a legume cover crop followed by a sequence of three cereal crops.

				Cereal crop sequence		
	Legume cover crop			Crop 1 (maize)	Crop 2 (rice)	Crop 3 (maize)
Species	Oct. 1993	Jan. 1994	July 1994	Dec. 1994	Apr. 1995	Sept. 1995
	kg N ha^{-1}					
Desmodium		19a‡	33b	38a	24a	35a
Pueraria		17a	48a	43a	26a	31a
Cleared native/fallow	98†	18a	25b	33a	22a	36a
CV, %		21	26	21	21	44

† Initial total inorganic N level (average of microplots) just prior to planting legume cover crop.
‡ Means with the same letter within a date are not significantly different ($P = 0.05$) using Fisher's LSD.

occurred even though the amount of N supplied by the shoot N source was 54 kg ha^{-1} less than that of *Desmodium*. Differences in the chemical composition of the legume shoots (Table 1) may have contributed initially to greater net mineralization of *Pueraria* shoot N. Residue decomposition and subsequent N mineralization are often enhanced when tissues have high N concentrations (low C:N ratio) and low concentrations of lignin and polyphenols. Others have found when residue N concentration exceeded 17.0 g kg^{-1}, net N mineralization occurred readily (Aber and Melillo, 1980; Constantinides and Fownes, 1994). The high N concentration (low C:N ratio) of *Pueraria* compared with that of *Desmodium* would likely have a greater potential for net N mineralization. In addition, N mineralization of legume leaf tissue has correlated well with soluble polyphenol concentration and polyphenol:N ratios <0.5 (Vallis and Jones, 1973; Palm and Sanchez, 1991; Oglesby and Fownes, 1992). Less polyphenols and a polyphenol:N ratio of 0.3 in *Pueraria* shoots would be consistent with initially greater N mineralization and accumulation of soil inorganic N than that of *Desmodium* shoots (polyphenol:N ratio of 0.8).

Contribution of Legume Nitrogen Sources to Soil Nitrogen Fractions

Amounts of inorganic and total N in the top 75 cm of the soil profile were measured after harvest of each crop grown in the cereal sequence. The relative contributions and amounts of soil organic N derived from the ^{15}N-labeled legume root-soil and shoot N sources were consistently greater than those recovered in the soil inorganic N fraction (Table 5). The percentages of the N derived from labeled legume shoot and root-soil sources and recovered in the soil N fractions was similar for both legume species. After harvest of the first cereal crop of maize, recoveries of legume-derived N in the soil inorganic N fraction appeared to decline from 59 to ≈14 g kg^{-1} after harvest of each of the last two crops in the cereal sequence. In contrast, during the sequence of three cereal crops, recoveries of legume-derived N in the soil organic N fraction appeared to remain constant at ≈160 g kg^{-1}. At the end of the three-cereal crop sequence, percentage recoveries of soil total N derived

Table 5. Recoveries of legume N sources as inorganic (NO_3 + NH_4) and organic N in the top 75 cm of soil after harvests of three sequential cereal crops preceded by a legume cover crop.†

^{15}N source	Legume cover crop	Inorganic	Organic	Total
		% ^{15}N recovery		
		Crop 1 (maize)		
Labeled shoot	*Desmodium*	1.9 (5.0)‡	16.3 (43.0)	18.2 (48.0)
	Pueraria	2.0 (3.8)	12.4 (23.9)	14.4 (27.7)
Labeled root-soil	*Desmodium*	13.9 (1.7)	18.4 (2.3)	32.3 (4.0)
	Pueraria	5.7 (0.8)	15.7 (2.2)	21.4 (3.0)
	CV, %	124	41	45
		Crop 2 (rice)		
Labeled shoot	*Desmodium*	0.5 (1.4)	12.4 (32.8)	12.9 (34.2)
	Pueraria	0.6 (1.1)	16.7 (32.1)	17.3 (33.2)
Labeled root-soil	*Desmodium*	3.5 (0.4)	18.9 (2.3)	22.4 (2.7)
	Pueraria	1.1 (0.1)	10.2 (1.4)	11.2 (1.5)
	CV, %	101	54	57
		Crop 3 (maize)		
Labeled shoot	*Desmodium*	0.4 (1.1)	14.0 (37.4)	14.4 (38.5)
	Pueraria	0.5 (0.9)	8.5 (16.3)	9.0 (17.2)
Labeled root-soil	*Desmodium*	3.3 (0.4)	34.2 (4.2)	37.5 (4.6)
	Pueraria	1.1 (0.2)	15.1 (2.1)	16.2 (2.3)
	CV, %	110	127	123

† All % ^{15}N recovery interaction (Legume × N source) and main effect means within a column of each crop were not significantly different ($P \leq 0.05$), except for Crop 2 inorganic N means of ^{15}N source effect.
‡ Values in parentheses are kg N ha^{-1} recovered from N initially present in each legume source.

from the legume shoot N sources (*Pueraria*, 90 g kg^{-1}; *Desmodium*, 140 g kg^{-1}) were not significantly different from the root-soil N source (*Pueraria*, 160 g kg^{-1}; *Desmodium*, 380 g kg^{-1}). The chemical composition traits of *Desmodium* (Table 1) that would make it more resistant than *Pueraria* to decomposition and N mineralization had no apparent effect on the percentage contribution of the N sources into the soil N pool. However, amounts of legume shoot N recovered in the soil N fractions of *Desmodium* microplots appeared to be greater than those of *Pueraria* microplots, because the amount of the shoot N source supplied by *Desmodium* (316 kg ha^{-1}) exceeded that of *Pueraria* (262 kg ha^{-1}).

Contribution of Legume Nitrogen Sources to the Following Cereal Crops

Because fertilizer N was not used, N available to the cereal crops would be derived from mineralization of soil organic matter, mineralization of legume mulches, and any N associated with precipitation and dry deposition. High elevation tropical rain forests can receive as much as 18 kg N ha^{-1} annually in bulk precipitation (Veneklaas, 1990). Aboveground dry matter and total N accumulation (Table 6) by the first cereal crop of maize after a cover crop of *Desmodium* (3.2 Mg ha^{-1}, 57 kg N ha^{-1}) was not significantly different than that of *Pueraria* (4.8 Mg ha^{-1}, 57 kg N ha^{-1}), even though plots with *Desmodium* had residues containing nearly 52 kg N ha^{-1} more than that of *Pueraria*. Significant differences in these traits due to a ^{15}N-labeled N source were unexpected and have no reasonable cause. The overall mean grain yield of 1.7 Mg ha^{-1} (data not shown) was apparently less than the local average yield of 2.3 Mg ha^{-1} and was probably affected by low rainfall during the growing season (July to November 1994 rainfall equaled only 75 cm; 49% less than the 11-yr average of 146 cm). For the second crop of rice and the third crop of maize in the cereal sequence, aboveground dry matter and N accumulation were unaffected by species of the legume cover crop and N source (Table 6). In contrast to the first crop of maize, the third cereal crop of maize in the sequence had 1.9-fold more dry matter and 1.5-fold more N accumulation. This greater response for dry matter and N accumulation was most likely the consequence of increased amounts of K, P, Ca, and Mg fertilizer used and a more favorable climate (Fig. 1) during the cropping cycle of the third cereal crop.

Cereal crop accumulation of legume N derived from the root-soil N source was unaffected by legume species and appeared to decrease after the first crop of maize in the cereal sequence (Table 6). Averaged across species, the legume root-soil N source contributed 50 g kg^{-1}

Table 6. Total aboveground dry mater (grain + vegetative), N accumulation, and N derived from legume cover crop N sources following harvest of three cereal crops grown in sequence.

	Cereal crop sequence								
	Crop 1 (maize)			Crop 2 (rice)			Crop 3 (maize)		
Legume cover crop	Aboveground dry matter	N content	N from cover crop	Aboveground dry matter	N content	N from cover crop	Aboveground dry matter	N content	N from cover crop
	kg ha^{-1}								
				Labeled shoot					
Desmodium	2900	61	11.5	2900	43	9.0a†	7700	89	9.9
Pueraria	4300	42	11.4	3300	38	5.1b	7500	83	6.3
				Labeled root-soil					
Desmodium	3500	52	3.2	2800	40	1.0c	7300	83	1.9
Pueraria	5300	71	2.8	3500	39	0.6c	7900	82	0.8
ANOVA source of variation									
Legume (L)	ns‡	ns	ns	ns	ns	*	ns	ns	ns
N source (N)	*	*	***	ns	ns	***	ns	ns	**
L × N	ns	ns	ns	ns	ns	*	ns	ns	ns
CV, %	30	41	35	18	12	31	14	24	56

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
† Means within a column followed by the same letter are not significantly different ($P = 0.05$) using Fisher's LSD.
‡ ns = not significant.

of the total N in the first crop of maize to no >20 g kg^{-1} of the total N accumulated by each of the following two cereal crops. Because the legume shoot N source contained substantially more N (Table 3), the contribution of legume shoot N to total N accumulated by each cereal crop was considerably greater ($P \leq 0.01$) than that of the legume root-soil N source (Table 6). Legume shoot N contributed ≈230 g kg^{-1} of the total N in the first maize crop. Subsequent contributions from legume shoot N declined to ≈170 g kg^{-1} for the second cereal crop of rice, and finally to ≈90 g kg^{-1} for the last crop of maize.

These results demonstrate that degradation of legume residues, particularly legume shoots, make a meaningful contribution to the N economy of cereal crops grown in the tropics. A substantial contribution (280 g kg^{-1}, root + shoot sources) to the N needs of cereals is realized for the crop immediately following the legume cover crop and extends even to the third cereal crop (110 g kg^{-1}) grown in a sequence across a 15-mo period.

Recoveries of Legume Nitrogen Sources

Although the chemical composition of the legume shoots differed (Table 1), an expected greater mineralization of *Pueraria* shoot residue N was not obvious as a greater N recovery by the cereal crops. Between 19 and 43 g kg^{-1} of the N initially contained in the legume shoot N sources of *Desmodium* and *Pueraria* was accumulated by each of the grain crops in the cereal sequence (Table 7). Differences in recoveries of N derived from the shoot N sources of the two legumes were not significant and totaled <100 g kg^{-1} of the N initially supplied. The actual N fertilizer value of mulches can be reduced by losses of N as the mulch decomposes. Up to 400 g kg^{-1} of the N in low C:N mulches can be lost as NH_3 (Larsson, et al., 1998). In contrast, the percentages of N recovered from the legume root-soil N source were substantially greater ($P \leq 0.01$) than those from the residues of legume shoots (Table 7). Total recovery of the legume root-soil N source by the three cereal crops was 280 g kg^{-1} for *Pueraria* and 490 g kg^{-1} for *Desmodium*. Even though a greater percentage of the N initially present in the legume root-soil N source was recovered in the cereal crops, the actual contribution of the root-soil N source to the N nutrition of the cereal crops was much less than the shoot N source (Table 6). At most, the legume root-soil N source contributed 6.1 kg N ha^{-1}, while as much as 30.4 kg N ha^{-1} was contributed by the shoot N source.

Mean total N recoveries for the ^{15}N-labeled legume N sources in the three cereal crops plus top 75 cm of the soil profile was 210 g kg^{-1} for the shoot N source and 660 g kg^{-1} for the root-soil N source (Table 7). These results demonstrate the largest source of legume N for the cereal crops was derived from legume shoots, primarily because of the abundance of N contained by the legume shoots placed on the soil surface. The legumes (shoot + root) provided an important source of N to the cereal crop sequence, contributing between 152 (*Pueraria*) and 198 g kg^{-1} (*Desmodium*) of the total aboveground N accumulated by the three cereal crops. The contribution of legume N continued to be detected even with the last crop of maize in the three-cereal crop sequence, but was minor compared with the contribution of N derived from the total native soil N pool of 8250 kg N ha^{-1}.

Regardless of cover crop species, legume shoots were substantially greater contributors of N to the cereal cropping sequence than were the root-soil N sources. Composition differences between the two legumes, most notably the C:N ratio, were expected to result in more N provided by *Pueraria* than *Desmodium*. In a decomposition study using mesh bags, Luna-Orea et al. (1996) estimated that ≈800 g kg^{-1} of the initial N in *Pueraria* residue was released during the first 12 wk compared with 550 g kg^{-1} for *Desmodium*. In the present study, however, the greater N content of *Desmodium* offset the potentially faster N mineralization rate of *Pueraria*. Nevertheless, cereal crop ^{15}N recovery values suggest considerable overestimation of N contributions from cover crop residues based on a mesh bag technique.

Table 7. Summary of ^{15}N recoveries by a sequence of three cereal crops following a legume cover crop with ^{15}N-labeled shoot and root-soil components.

		Cereal crop sequence				
^{15}N source	Legume cover crop	Crop 1 (maize)†	Crop 2 (rice)	Crop 3 (maize)	Cereal crop total	Total (crops + soil)‡
		% ^{15}N recovery				
Labeled shoot	*Desmodium*	3.6	2.9	3.1b§	9.6c	24.0
	Pueraria	4.3	1.9	2.4b	8.6c	17.6
Labeled root-soil	*Desmodium*	25.7	8.4	15.3a	49.4a	86.9
	Pueraria	19.1	3.8	5.1b	28.0b	44.2
Source of variation		ANOVA				
Legume (L)		ns¶	*	*	**	ns
N source (N)		***	**	**	***	*
L × N		ns	ns	*	*	ns
CV, %		41	42	43	32	63

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
† Values are for total aboveground plant tissues (grain + vegetative) at harvest.
‡ Values are for the sum of the three cereal crops plus total N (inorganic + organic) in the top 75 cm of soil after harvesting the final crop in the cereal sequence.
§ Means within a column followed by the same letter are not significantly different ($P = 0.05$) using Fisher's LSD.
¶ ns = not significant.

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